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(54) Title: NOVEL BRANCHED HYBRID AND CLUSTER PEPTIDES EFFECTIVE IN DIAGNOSING AND DETECTING NON-A, NON-B HEPATITIS

(57) Abstract

The present invention relates to novel branched peptides specific for the diagnosis and prevention of non-A, non-B hepatitis (NANBH), as well as hepatitis C virus (HCV) infection. More particularly, the present invention is directed to branched synthetic substituted and hybrid peptides containing at least one epitope which is effective in detecting NANBH-associated antibodies in patients with NANBH using immunoassay techniques. In addition, this invention provides immunoassays and kits for the detection and diagnosis of NANBH or HCV infection using the subject peptides.

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NOVEL BRANCHED HYBRID AND CLUSTER PEPTIDES EFFECTIVE IN DIAGNOSING AND DETECTING NON-A, NON-B HEPATITIS

The present invention relates to novel branching peptides specific for the diagnosis and prevention of non-A, non-B hepatitis (NANBH), including hepatitis C virus (HCV) infection. More particularly, the present invention is directed to branched synthetic peptides containing at least one epitope which is effective in detecting NANBH-associated antibodies in patients with NANBH using immunoassay techniques. Further, the present invention is directed to synthetic peptides which are hybrids of the peptides described herein.

Non-A, non-B hepatitis (NANBH) remains the most common form of post-transfusion hepatitis, imposing a strong need for sensitive and specific diagnostic screening methods to identify potential blood donors and other persons who may be carriers of the disease. Thus, accurate screening methods are needed to permit removal of contaminated blood and blood products from the blood supply with a high degree confidence.

The etiological agent of NANBH, HCV, has been cloned and identified by several groups [Houghton et al., EP 0318216, published 5/1989; Okamoto et al. (1990) Jpn. J. Exp. Med. 60:167; Houghton et al., EP 0388232, published 9/1990; and Kato et al. (1990) Proc. Natl. Acad. Sci. USA 87:9524; Arima et al. (1989a) Gastroenterologia Japonica 24:540; Reyes et al. (1990) Science 247:1335; Arima et al. (1989b) Gastroenterologia Japonica 24:545; Maeno et al. (1990) Nucleic Acids Res. 18:2685]. The HCV genome is about 10 kilobases (kb) in length and encodes a single polyprotein which is

processed into structural and non-structural proteins. From the N terminus, the polyprotein includes the capsid and envelope proteins of the structural region and the NS-1 to NS-5 proteins of the non-structural region.

While some of the antigenic regions of HCV have been identified, peptides and recombinant proteins from these regions exhibit a variable degree of sensitivity and selectivity in detection and diagnosis of NANBH carriers. Antigenic regions have been reported in the core, or capsid, protein [Hosein et al. (1991) Proc. Natl. Acad. Sci. USA 88:3647; UBI HCV EIA Product Insert (1990); Okamoto et al. (1990) Jap. J. Exp. Med. 60:223; U.S. Patent No. 5,106,726; Takahashi et al. (1992) J. Gen. Virol. 73:667; Kotwal et al. (1992) Proc. Natl. Acad. Sci. USA 89:4486]; in the envelope, NS-1, NS-2 and NS-3 proteins [Wang et al., EP 0468527, published Jan. 29, 1992]; NS-4 protein [Houghton (1989); Kuo et al. (1989) Science 244:362; U.S. Patent No. 5,106,726] and NS-5 protein [Maeno et al. (1990) Nucleic Acids Res. 18:2685; Wang (1992)].

In addition to HCV-derived antigens, there exist other NANBH-associated antigens that appear to be encoded by a host cellular sequence. One such antigen, known as the GOR epitope, is reactive with sera from individuals who are PCR positive for HCV [Mishiro et al. (1990) Lancet 336:1400].

Serological validation has been used to map epitopes within certain HCV antigenic regions as described in Wang (1992) and U.S. Patent No. 5,106,726, each of which is incorporated herein by reference. These mapping studies employed synthetic peptides to screen well-characterized NANBH serum panels and

permitted identification of strong HCV antigens. Further refinement of the epitope analysis using serological validation techniques has led to the discovery that small clusters of amino acid residues contained within longer branched peptides or fusions of peptides containing one or more epitopes from separate regions of the HCV genome provide a superior and more sensitive assay for diagnosis and detection of NANBH carriers as well as for HCV infection. Hence, the present invention permits earlier detection of NANBH seroconversion and shows improved specificity, for example, fewer false positive serum samples are detected.

The present invention relates to branched synthetic peptides for the diagnosis and detection of NANBH and HCV infection. In particular the subject peptides are provided as a peptide composition having at least one branched peptide represented by the formula

$(peptide)_2X$

$(peptide)_4X_2X$

$(peptide)_8X_4X_2X$

$(peptide)_{16}X_8X_4X_2X$

where X is an amino acid or an amino acid analog having two amino groups and one carboxyl group with each group being capable of forming a peptide bond linkage, and where the peptide moiety comprises at least one epitope which is specifically immunoreactive with antibodies against HCV. The peptide moiety further comprises at least one cluster of from about 3 to about 20 contiguous amino acids from the sequences:

Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Leu-His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly, (Pep3; SEQ ID NO:1),

Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala, (Pep8; SEQ ID NO:2),

Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr, (Pep11; SEQ ID NO:3),

Glu-Ile-Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-Gly-Gly-Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Lys-Cys-Asp-Glu-Leu-Ala-Ala-Lys-Leu-Val-Ala-Leu, (Pep18; SEQ ID NO:4),

Pro-Val-Val-Pro-Gln-Ser-Phe-Gln-Val-Ala-His-Leu-His-Ala-Pro-Thr-Gly-Ser-Gly-Lys-Ser, (Pep25; SEQ ID NO:5)

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu, (IIH; SEQ ID NO:6),

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile, (IIID; SEQ ID NO:7),

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe, (V; SEQ ID NO:8),

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg, (VIIIE; SEQ ID NO:9),

Asn-Asp-Arg-Val-Val-Val-Ala-Pro-Asp-Arg-Glu-Ile-Leu-Tyr-Glu-Ala-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ala-Ser-Lys-Ala-Ala-Leu-Ile-Glu-Glu-Gly-Gln-Arg-Met-Ala-Glu-Met-Leu-Lys-Ser-Lys-Ile-Gln-Gly-Leu, (PepA; SEQ ID NO:10),

or a sequence corresponding to one of these sequences which is from a corresponding region in a strain or isolate of HCV. Moreover, when the peptide moiety comprises two or more clusters, the clusters are joined by a linking group or when the clusters each have a sequence from a different one of the above sequences, then the clusters can be joined directly or joined by a linking group.

When the peptide moiety contains sequences from different ones of the above sequences, such peptides are referred to as hybrid peptides. Hybrid peptides can but do not necessarily contain clusters. Clusters in hybrid peptides can be joined directly or by linking groups. In the hybrid peptides, the length of contiguous amino acids from each of the sequences can be up to about 60 residues.

Another aspect of the invention provides a method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an immunoeffective amount of the subject peptide composition in an immunoassay procedure, and particularly in an ELISA procedure, or a passive hemagglutination (PHA) assay. Immunoassays and kits for the detection and diagnosis of NANBH and HCV infection are also provided.

In accordance with the present invention, extensive epitope analysis led to the refinement and further definition of epitopes that are useful in the detection and diagnosis of NANBH and HCV infection. This analysis has established that effective diagnostic peptides for NANBH or HCV infection are branched, synthetic peptides which are hybrids of peptides containing one or more HCV epitopes from different peptides, also referred to herein as hybrid peptides. Moreover, the peptides of this invention also include branched synthetic peptides having at least one epitope which is specifically immunoreactive with antibodies against HCV and having a peptide moiety which comprises one or more clusters of about 3 to about 20 contiguous amino acids from the peptides designated as Pep3, Pep8, Pep11, Pep18, Pep25, IIH, IIID, V, VIIIE, PepA, or a homologous peptide from a corresponding region in another strain or isolate of HCV. In addition, when the peptide moiety of these peptides, also referred to herein as cluster peptides, contain two or more clusters, then the clusters are joined by a linking group. The linking group consists of, but is not limited to, one or more naturally occurring amino acids, one or more unnatural amino acids, or one or more amino

acid analogues which can form peptidyl bonds (or peptidyl-like bonds) and are stable to the conditions employed during peptide synthesis. In the case of hybrid peptides that contain clusters, the clusters can be joined directly or can be joined by a linking group.

The sequences of the peptides subjected to detailed epitope analysis, and from which the peptide moieties of the subject branched peptides are derived, are set forth above and are the sequences designated as Pep3, Pep8, Pep11, Pep18, Pep25, IIH, IIID, V, VIIIE and PepA or a homologous peptide from the corresponding region in another strain or isolate of HCV, and analogues and segments thereof.

As used herein a "cluster" is a sequence from 3 to about 20 contiguous amino acids from one of the peptide sequences described herein or an analog or segment thereof. In a preferred embodiment, a cluster has a sequence of 3 to 9 contiguous amino acids.

The branched hybrid and cluster peptides of the present invention including their analogues and segments are useful for the detection of antibodies to HCV in body fluids, the diagnosis of NANBH, and for the vaccination of healthy mammals, particularly humans, to stimulate the production of antibodies to HCV, including neutralizing or protective antibodies.

The subject branched peptides can comprise combinations or segments, i.e., longer or shorter peptide chains by having more amino acids, including unnatural amino acids, added to the terminal amino acids, or by having amino acids removed from either terminal end. For example, the sequence KKK (Lys-Lys-Lys) can be added to the amino terminus of peptides.

Similarly, an M (methionine) residue can be placed at the carboxy terminus of th p ptide moiety, i.e. between the peptide moiety and the branch structure.

As used herein "segments" means a shorter region of a parent peptide which retains an epitope effective in detecting NANBH-associated antibodies. For example, C10A is a segment of VIIIE, its parent peptide. A segment can be derived from either end of its parent peptide or from an internal sequence of its parent peptide.

The subject branched peptides can also comprise analogues thereof to accommodate strain-to-strain variation among different isolates of HCV or other substitutions in the prescribed sequences which do not effect immunogenicity of the epitope. HCV is indicated to have frequent mutations. Several variant strains/isolates are known to exist, such as PT, J, J1 and J4 [Houghton, 1989; Okamoto, 1990; Houghton, 1990; and Kato, 1990] and it is expected that other variant strains also exist. Adjustments for conservative substitutions and selection among the alternatives where non-conservative substitutions are involved, can be made in the prescribed sequences. The analogues of the branched synthetic peptides, especially the hybrid peptides, can therefore comprise substitutions, insertions and/or deletions of the recited amino acids of the above sequence to accommodate the various strains, as long as the immunoreactivity recognizable by the antibodies to HCV is preserved. The substitutions and insertions can be accomplished with naturally-occurring amino acids, unnatural amino acids or amino acid analogues capable of forming peptidyl bonds or

peptide-like bonds (e.g., peptide thiol analogues). Analog peptides in accordance with this invention are synthesized and tested against an HCV serum panel to determine the immunoreactivity of the peptide as described hereinbelow.

Further, with appropriate amino acid modification or substitutions, it is expected that various peptide analogues based on the prescribed amino acid sequences can be synthesized with properties giving rise to lower background readings or better binding capacity to solid phases useful for HCV antibody screening assays. In particular, peptides containing unnatural amino acids can significantly reduce background readings.

The subject branched peptides can also be used to form conjugates, i.e., the peptides can be coupled directly or indirectly, by methods known in the art, to carrier proteins such as bovine serum albumin (BSA), human serum albumin (HSA), or to red blood cells or latex particles.

As used herein, natural amino acids are the 20 amino acids commonly found in proteins (i.e. alanine, aspartic acid, asparagine, arginine, cysteine, glycine, glutamine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan and valine). As used herein the natural amino acids also include the D- and L- forms of such amino acids.

As used herein "unnatural amino acids" include both D- and L- forms of any other amino acids whether found in a protein, whether found in nature or whether synthetically produced. Unnatural amino acids can

include, but are not limited to, ß-alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine, gamma-amino butyric acid, homoserine, citrulline and the like.

The branched peptides of the present invention are represented by one of the formulae:

$(peptide)_2X$

$(peptide)_4X_2X$

$(peptide)_8X_4X_2X$

$(peptide)_{15}X_8X_4X_2X$

wherein X is an amino acid or an amino acid analog having two amino groups and one carboxyl group, each group capable of forming a peptide bond linkage. Preferably X is lysine or a lysine analog such as ornithine. The amino acid analog can be an α-amino acid, a ß-amino acid, or any other either natural or non-natural amino acid with two amino groups and one carboxyl group available for forming peptide bonds. Preferred branched peptides of the invention are dimers, tetramers and octamers, especially those having a branching core structure composed of lysine, i.e. where X is lysine. Branched dimer are especially preferred.

The peptide moiety of the branched peptides can vary in length from about 10 to about 100 amino acids residues. Preferably the peptide moieties contain about 17 to about 60 amino acid residues. Moreover, the hybrid and cluster peptide moieties can be optimized to the minimal overall length necessary to contain an epitope effective in detecting NANBH-associated antibodies yet still retain the superior sensitivity and selectivity of the present invention.

The preferred branched peptides of the present invention are provided in Table 1. The source of each peptide is provided in Table 2.

TABLE 1
BRANCHED PEPTIDES[a,b]

HYBRID PEPTIDES WITH OR WITHOUT CLUSTERS	
B1	YEPPVVHGCPLPPPKSPPVPPPRKKRTIIPDREVLYREFDEMEECSQHLPYIPKPQRKTKRNTNRRPQDVKFPGGGQIVG-DIM
B2A	LYREFDEMEDCSQHLPYIPKPNRKTKRNTQRRPNDVKFPGGGNIVGM-OCT
B2B	PDREILYREFDEMEDCSQHLPYIPKPNRKTKRNTQRRPNDVKFPGGGNIVGM-OCT
B2C	IIPDREILYREFDEMEDCSQHLPYIPKPNRKTKRNTQRRPNDVKFPGGGNIVGM-OCT
B2CK	IIPDREILYREFDEMEDCSQHLPYIPKPNRKTKRNTQRRPNDVKFPGGGNIVGK-OCT
B2D	SGKPAIIPDREILYREFDEMEDCSQHLPYIPKPNRKTKRNTQRRPNDVKFPGGGNIVGM-OCT
B2DK	SSKPAIIPDREILYREFDEMEDCSQHLPYIPKPNRKTKRNTQRRPNDVKFPGGGNIVGK-OCT
B3	GC*SGG*TYDIIICDELHSTDATSIVG1GTILDQAETAGRHLIFCHTKKKCDELASKLVALGM-OCT
B4A	YEPPVVHGRHLIPCHTKKKCDELASKLVALGM-OCT
B4B	PLVETWKKPDYEPPVVHGRHLIPCHTKKKCDELASKLVALGM-OCT
B6A	IEQGMMLAENFKQKALGLPRRGPRLGLRATRKTTERSQPRGRM-OCT
B6B	SGKPAIIPEREVIEQGMMLAENFKQKALGLPRRGPRLGLRATRKTTERSQPRGRM-OCT
B7	SGKPTIIPDREILYREFDEMEDCSQHLPYIDQGMMLAENFKQKALGLVKFPGGGQI-DIM
3KH7	KKKSGKPTIIPDREILYREFDEMEDCSQHLPYIDQGMMLAENFKQKALGLVKFPGGGQI-DIM
CLUSTER PEPTIDES	
C1A	IIPDREILYREFDEMEDCSQHLPYI-DIM
C1B	SSKPAIIPDREILYREFDEMEDCSQHLPYI-DIM
C2A	PLVETWKRPDYEPPVVH-OCT
C2B	PLVETWKKPDYEPPVVH-OCT
C3	KKKSGKPTIIPDREILYREFDEMEDCSQHLPYIDQGMMLAENFKQKALGL-DIM
C4	KKRIPKPNRKTKRNTQRRPNDVKFPGGGNIVGGVYLVPRRGPRLGLRATRKTTERSQPRGRR-DIM
C5A	DCSQHLPYIDQGMMLA-DIM
C5B	ILYREFDEMEDCSQHLPYIDQGMMLA-DIM
C5C	SGKPTIIPDREILYREFDEMEDCSQHLPYIDQGMMLA-DIM
3KC5C	KKKSGKPTIIPDREILYREFDEMEDCSQHLPYIDQGMMLA-DIM
C6A	PLVETWKKPEYEPPVVH-DIM
C6B	PLVETWKKPEYEPPVVH-OCT

C7A	CSQHvPYIEQGMlLAEQFKQKAvGL-DIM
C7B	LYREFDElEECSQHvPYIEQGMlLAEQFKQKAvGL-DIM
C7C	SGKPAvIPDREvLYREFDElEECSQHvPYIEQGMlLAEQFKQKAvGL-DIM
3KC7C	KKKSGKPAvIPDREvLYREFDElEECSQHvPYIEQGMlLAEQFKQKAvGL-DIM
C8A	DYEPPVVH-DIM
C8B	PLVETWKKpDYEPPVVH-DIM
C8C	PLVETWKoPDYEPPVVH-DIM
C9A	GRHLIvCHSKKKCDElAAKLVALG-DIM
C9B	EIPFYGKAvPLEvIKGGRHLIvCHSKKKCDElAAKLVALG-DIM
C10A	RPNDvKFPGGGNIvGGVYLVPRRGPRlGLRATRKTTERSQpRGRR-DIM
C10B	IPKPNRKTKRNTQRRPNDvKFPGGGNIvGGVYLVPRRGPRlGLRATRKTTERSQpRGRR-DIM
3KC10B	KKKIPKPNRKTKRNTQRRPNDvKFPGGGNIvGGVYLVPRRGPRlGLRATRKTTERSQpRGRR-DIM

[a] Abbreviations: The amino acid sequences are provided in one letter code except that unnatural amino acids are indicated by: v, norvaline; l, norleucine; p, hydroxyproline; o, ornithine. Other abbreviations are DIM, lysine dimer; OCT, lysine octamer.

[b] The branched core for these peptides is composed of lysine residues, e.g., 1 lysine for dimer peptides and 7 lysines for octamer peptides.

TABLE 2

SOURCE OF HYBRID AND CLUSTER BRANCHED PEPTIDES

Source Peptide	Branched Peptides from Tabl 1
Pep11	C2A,C2B,C6A,C6B,C8A,C8B,C8C
Pep18	C9A,C9B
IIH	C3,C5A,C5B,C5C,3KC5C,C7A,C7B,C7C,3KC7C
IIID	C1A,C1B
VIIIE	C4,C10A,C10B,3KC10B
Pep3 + Pep18	H3
Pep11 + Pep18	H4A,H4B
Pep11 + IIID + VIIIE	H1
IIH +VIIIE	H6A,H6B,H7,3KH7
IIID + VIIIE	H2A,H2B,H2C,H2CK,H2D,H2DK

The peptide compositions of the present invention can be composed of one or more of the branched hybrid peptides, branched cluster peptides or any combination of such peptides. Preferably such compositions contain from one to 10 branched peptides, and even more preferably from one to four branched peptides.

In a preferred embodiment, the peptide compositions of the present invention can be a mixture of branched peptides (1) C3 dimer, C9B dimer, C6A dimer and 3KH7 dimer; (2) 3K204h dimer, C4 dimer, C2B octamer; (3) C4 dimer, C9B dimer, C6A dimer and H7 dimer; or (4) 3KH7 dimer, C6A dimer and C4 dimer. The effective ratio of peptides for diagnosing or detecting NANBH or HCV present in peptide compositions containing mixtures of the subject peptides can be readily determined by one of ordinary skill in the art. Typically, these ratios range from about 1 to about 50 on a per weight basis of peptide.

An especially preferred peptide composition for diagnosis and detection of NANBH or HCV infection is mixture (1), branched peptides 3KC10B dimer, C9B dimer, C6A dimer and 3KH7 dimer in a weight ratio of 5:15:1:25.

To determine the efficacy of the subject peptides in detecting and diagnosing NANBH and HCV infection, the peptides are tested for their immunoreactivity with special specimens previously selected through the screening of thousands of patient and normal sera for immunoreactivity with HCV. Such serum panels are commercially available and examples thereof are provided in the Examples.

The strategy for serological validation depends on the expected characteristics of the targ t epitopes. For example, universal immunodominant epitopes, such as the gp41 transmembrane peptide of HIV-1, can be screened by a single representative serum sample from a patient known to be infected with the virus. Epitopes that are not recognized by all infected individuals, or those for which antibody is produced late or only transiently, and especially epitopes which give rise to neutralizing antibodies, must be screened by large panels of sera. While both methods of screening can be employed in the present invention to refine the epitope analysis of HCV using the subject peptides, the latter method is particularly useful in assessing the subject peptides for superior selectivity and sensitivity.

The identification of the immunoreactive epitopes is also dependent on the panel of sera used. The more closely the panel represents the population most likely to be seropositive for an epitope, the greater the chance that the epitope will be identified and thoroughly mapped. Hence, to extend the range of reactivity of an assay comprised of previously identified epitopes, a large number of samples from individuals at risk of infection but seronegative against known epitopes should be employed for screening.

The process of "serological validation" is particularly difficult when the epitopes to be identified elicit antibodies only in a subpopulation of an infected patient group. When such epitopes become targets for identification, special attention must be paid to synthetic peptides which show very weak

reactivity when tested by an enzyme immunoassay or any other immunological testing method.

In this regard, the low background absorbance of synthetic peptides, especially peptides with unnatural amino acids, allows for the precise detection of weak reactivities. In some cases, absorbances of 50 mA versus background reading are of sufficient significance and can lead to the identification of important epitopes through successive refinement of the amino acid sequence of a peptide. With good laboratory practices, consistent and reliable results can be obtained when working in the range of absorbances below 200-300 mA.

The advantages of using synthetic peptides are known. Since the peptides not derived biologically from the virus, there is no danger of exposure to a disease causing pathogen. The peptides can be readily synthesized using standard techniques, such as the Merrifield method of synthesis [Merrifield (1963) J. Am. Chem. Soc. 85:2149-2154]. Hence, there is no involvement with HCV at any time during the process of making the test reagent. Another problem which can be minimized by using peptides rather than recombinantly expressed proteins (or peptides) is the rate of false positive results caused by the presence of antigenic material co-purified with the HCV fusion protein. For example, certain normal individuals have antibodies to Escherichia coli or yeast proteins which are cross reactive with the antigenic materials from the expression system used in recombinant-based diagnostic tests. Sera from such normal individuals show a false

positive reaction in such immunoassays which is eliminated in immunoassays of the present invention.

Moreover, because the peptide compositions of the present invention are synthetically prepared, the quality can be controlled and as a result, reproducibility of the test results can be assured. Also, since very small amounts of a peptide are required for each test procedure, and because the expense of preparing a peptide is relatively low, the cost of screening body fluids for antibodies to HCV and the diagnosis of NANBH infection is relatively low.

The peptides and peptide compositions prepared in accordance with the present invention can be used to detect HCV infection and diagnose NANBH by using them as the test reagent in an enzyme-linked immunoadsorbent assay (ELISA), an enzyme immunodot assay, a passive hemagglutination assay (e.g., PHA test) or other well-known immunoassays. In accordance with the present invention, any suitable immunoassay can be used with the subject peptides. Such techniques are well known to the ordinarily skilled artisan and have been described in many standard immunology manuals and texts, see for example, by Harlow et al. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 726 pp. In a preferred embodiment, the immunoassay is an ELISA using a solid phase coated with the peptide compositions of the present invention. ELISA techniques are well known in the art. In another preferred embodiment the immunoassay is a PHA assay.

The immunoassays of the present invention are used to screen body fluids and tissues for the presence

of NANBH or HCV and thereby to detect such agents and aid the practitioner in diagnosis of NANBH or HCV infection. The body fluids which can be subjected to such screening include blood and blood fractions (e.g. serum), saliva, or any other fluid which contains antibodies against HCV.

Another aspect of the present invention is directed to a kit for the detection and diagnosis of NANBH or HCV infection in mammalian body fluids (e.g. serum, tissue extracts, tissue fluids), in vitro cell culture supernatants, and cell lysates. The kit can be compartmentalized to receive a first container adapted to contain one or more of the peptides (i.e. a peptide composition) of this invention.

Preferably the kit of this invention is an ELISA or a PHA test kit for detection or diagnosis of NANBH or HCV infection. For an ELISA test kit, the kit contains (a) a container (e.g., a 96-well plate) having a solid phase coated with one of the subject peptide compositions; (b) a negative control sample; (c) a positive control sample; (d) specimen diluent and (e) antibodies to human IgG, which antibodies are labelled with a reporter molecule. If the reporter molecule is an enzyme, then the kit also contains a substrate for said enzyme.

In an exemplified use of the subject kit, a sample to be tested is contacted with a mammalian body fluid, diluted in sample diluent if necessary, for a time and under conditions for any antibodies, if present, to bind to the peptide contained in the container. After removal of unbound material (e.g. by washing with sterile phosphate buffered saline), the

secondary complex is contacted with labelled antibodies to human IgG. These antibodies bind to the secondary complex to form a tertiary complex and, since the second antibodies are labeled with a reporter molecule, when subjected to a detecting means, the tertiary complex is detected. The reporter molecule can be an enzyme, radioisotope, fluorophore, bioluminescent molecule, chemiluminescent molecule, biotin, avidin, streptavidin or the like. For ELISA the reporter is preferably an enzyme.

The examples serve to illustrate the present invention and are not to be used to limit the scope of the invention.

EXAMPLE 1

Detection of antibodies to the core region of HCV in early seroconversion sample using branched cluster peptides

The wells of 96-well plates were coated separately for 1 hour at 37° with 1 μg/ml of peptide using 100 μL per well in 10mM $NaHCO_3$ buffer, pH 9.5, for each of two branched peptides from the core region of HCV (peptide C4, Table 1; and test peptide T1 related to VIIIE and having the sequence KKKIPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATRKTSERS QPRGRR-DIM

The peptide-coated wells were then incubated with 250 μL of 3% by weight of gelatin in PBS in 37°C for 1 hour to block non-specific protein binding sites, followed by three washes with PBS containing 0.05% by volume of TWEEN 20 and then dried. The test specimens containing HCV antibody positive patient sera were

diluted with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume TWEEN 20 at dilutions of 1:20 volume to volume, respectively. 200 μL of the diluted specimens were added to each of the wells and allowed to react for 15 minutes at 37°C.

The wells were then washed six times with 0.05% by volume TWEEN 20 in PBS in order to remove unbound antibodies. Horseradish peroxidase conjugated goat anti-human IgG was used as a second antibody tracer to bind with the HCV antibody-peptide antigen complex formed in positive wells. 100 μL of peroxidase labeled goat anti-human IgG at a dilution of 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN 20 in PBS was added to each well and incubated at 37°C for another 15 minutes.

The wells were washed six times with 0.05% by volume TWEEN 20 PBS to remove unbound antibody and reacted with 100 μL of the substrate mixture containing 0.04% by weight orthophenylenediamine (OPD) and 0.12% by volume hydrogen peroxide in sodium citrate buffer, pH 5.0. This substrate mixture was used to detect the peroxidase label by forming a colored product. Reactions were stopped by the addition of 100 μL of 1.0M H_2SO_4 and the A_{492}nm measured.

The sensitivity of these two peptides in detecting antibody to the core region was tested with a seroconversion panel in which the earliest antibody response is known to be against core (Serologicals Panel 4813, Donor 02190D as referenced in U.S. Patent No. 5,106,726; early core response as referenced in Hosein, 1991). The bleed date chosen for comparison was August 30, 1988. The optical density obtained with peptide C4

was 0.320 and with T1, 0.512. Both peptides were more sensitive than the linear peptide VIIIE with three lysine residues at its N terminus when coated at the same concentration, in which case the absorbance on the same sample was 0.075.

EXAMPLE 2

Branched hybrid peptides confer improved sensitivity and specificity relative to the individual peptides

The immunoreactivity of branched hybrid peptide 3KH7 (Table 1) containing an epitope from the NS-4 and core regions of HCV was tested on panel 3 containing 41 known NANBH samples using the ELISA assay format as described in Example 1. Table 3 shows that this hybrid peptide retained the reactivity of both the NS-4 and the core regions as compared to octamer T2 (related to VIIIE) from the core region only and peptide T3 (SEQ ID NO:11; related to IIH) from the NS-4 region only. Furthermore, sample 3-35 showed improved reactivity with the hybrid peptide relative to either single region peptide.

The specificity of the hybrid peptide 3KH7 was tested on a panel of 48 random blood donor samples screened negative for antibodies to HCV. Only one of the negative samples had an absorbance greater than 0.200 A with the hybrid peptide, whereas twenty percent of these samples had absorbance values greater than 0.200 A with the octamer T2. Branched cluster peptide C3, containing an epitope from the NS-4 region but lacking the core epitopes, gave absorbance values greater than 0.200 A on 5/48 negative samples. Therefore the combination of epitopes from the two

regions as presented in the hybrid peptide resulted in improved specificity for detection of NANBH.

Table 3

HCV Positive Sample[a]	A_{492nm} (pos/neg)		
	3KH7	T2[b]	T3[b]
3-2	0.491(+)	0.068(-)	0.756(+)
3-10	1.164(+)	0.027(-)	1.857(+)
3-21	2.576(+)	0.095(-)	2.226(+)
3-32	1.653(+)	1.188(+)	2.236(+)
3-35	2.303(+)	0.800(+)	0.324(+)
3-39	1.441(+)	0.486(+)	1.676(+)
3-7	1.118(+)	3.229(+)	0.582(+)
3-8	0.696(+)	1.860(+)	0.003(-)
3-9	1.408(+)	2.797(+)	0.163(-)
3-12	1.870(+)	0.328(+)	0.037(-)
3-26	1.607(+)	3.233(+)	0.355(+)

[a] The remaining samples in panel 3 were negative on all peptides or showed no improvement in using the branched hybrid peptide compared with the test peptides.

[b] The sequences of control peptides T2 and T3 are, respectively, VKFPGGGQIM-octamer and

KKKSGKPAIIPDREVLYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGL.

EXAMPLE 3

Comparison of sensitivity and specificity in detection of NANBH-associated antibodies in branched cluster peptides with unnatural amino acids linking groups

The immunoreactivity of branched cluster peptide C10B (Table 1) from the core region with clusters separated by unnatural amino acids was compared

with a similar peptide T1 (Example 1) lacking such unnatural amino acids, using panel 3 samples in an ELISA assay format as described in Example 1. Table 4 illustrates seven samples in which the absorbance for the peptide containing unnatural amino acids was higher than for the corresponding peptide lacking unnatural amino acids, i.e, branched peptide C10B was more sensitive than T1. The specificity of these two peptides was equivalent with 0/48 negative samples having absorbance readings greater than 0.200 A.

The immunoreactivity of branched cluster peptide C8C (Table 1) from the NS-5 region of HCV having clusters separated by unnatural amino acids was compared with the corresponding branched peptide lacking unnatural amino acids (C6A dimer; this peptide has clusters separated by natural amino acids; Table 1). Both peptides detected 18/41 samples from panel 3 as positive. Table 5 shows six samples in which the absorbance with the peptide containing unnatural amino acids was higher than for the corresponding peptide lacking unnatural amino acids.

Table 6 shows four reactive samples from panel 3 in which peptide 3KC7C (Table 1) had increased absorbance values compared to peptide C3 (Table 1), i.e., the presence of unnatural amino acids imparted greater sensitivity to the assay for detection of NANBH and HCV.

Furthermore, a marked improvement in specificity, measured by the ELISA procedure as described in Example 1, was also obtained with branched cluster peptide 3KC7C from the NS-4 region of HCV having clusters separated by unnatural amino acids. With

peptide 3KC7C, 0/48 negative samples had absorbance values greater than 0.200 A, whereas 5/48 had absorbance values greater than 0.200 A with branched peptide C3 which lacked unnatural amino acids but had natural amino acid separating the clusters. Specificity was also improved by addition of the unnatural amino acid in peptide C8C, in that only 1/48 negative random donor samples had absorbance readings greater than 0.200 A, compared with 2/48 for peptide C6A.

Table 4

HCV Positive Sample[a]	A_{492nm}	
	C10B	T1
3-7	2.451	2.005
3-8	1.081	0.873
3-9	2.665	2.272
3-12	0.446	0.352
3-24	2.378	2.088
3-25	2.399	1.555
3-39	1.289	0.767

[a] See Table 6

Table 5

HCV Positive Sample[a]	A_{492nm}	
	C8C	C6A
3-1	1.622	1.246
3-5	2.130	1.907
3-11	0.895	0.782
3-27	2.710	2.463
3-33	2.108	1.763
3-36	2.236	2.016

[a] See Table 6

Table 6

HCV Positive Sample[a]	A_{492nm}	
	3KC7C	C3
3-7	0.389	0.350
3-14	2.034	1.670
3-29	1.561	1.350
3-41	> 3.0	2.570

[a] For Tables 4-6, the remaining samples in panel 3 were negative on both peptides or showed no improvement in using the branched hybrid peptide compared to the test or control peptides.

EXAMPLE 4

Improved NS-5 immunorereactivity conferred by a shorter branched branched peptide relative to its linear parent peptide

A 17 residue branched octamer cluster peptide, C2A from the NS-5 region of HCV (Table 1), was able to detect antibody in all 23/41 samples from panel 3 that were reactive with its parent linear peptide T4, a 44 residue peptide having the sequence ARPDYNPPLVETWKKPDYYYEPPVVHGCPLPPPKSPPVPPPRKKRT SEQ ID NO:12). Table 7 shows five samples from panel 3 that exhibited higher absorbance values with peptide octamer C2A than with linear peptide T4.

Table 7

HCV Positive Sample[a]	A_{492nm}	
	T4	C2A
3-7	0.742	1.377
3-11	1.188	1.815
3-16	3.139	3.745
3-26	2.263	2.527
3-33	2.118	2.631

[a] The remaining samples in panel 3 were negative on both peptides or showed no improvement in using 17-mer compared with the 44-mer.

EXAMPLE 5

Earlier detection of NANBH-associated antibodies in a seroconversion panel using a mixture of branched peptides

A mixture of dimer peptides, 3KC10B, 3KH7, C9B and C6A (1, 5, 3, 0.25 ug/ml, respectively) was coated on wells of 96-well plates and assayed using the ELISA procedure described in Example 1. The sequence of each branched peptide is provided in Table 1. The sensitivity of this mixture was compared with that of Format C peptides (described in EPO 0468527 A2 and consisting of peptides IIH, V and VIIIE coated at 5, 3 and 2 μg/ml, respectively) using seroconversion panel 4813 described in Example 1. Table 8 shows that seroconversion samples were consistently positive on the mixture of peptides one week before antibody was detected by Format C. Earlier samples at bleed dates of August 9 and August 16, 1988 show fluctuation of

antibody response near the cutoff of the assay and indicate detection of passive antibodies from the transfusion of this patient that occurred July 19, 1988.

Table 8

Panel	Donor	Bleed Date	ALT[a] (u/L)	EIA Ratio	
				Format C	Mixture[b]
1	02190D	880809	40.0	0.108	1.197
		880816	32.0	0.045	0.899
		880823	32.0	0.025	1.044
		880830	180.0	1.037	1.197
		880928	401.0	7.193	3.303
		881109	NA	10.185	10.250
		881122	NA	9.770	11.548

[a] Abbreviations: ALT = Alanine amino-transferase
[b] The composition of Format C and Mixture are described in Example 5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: United Biomedical Inc.

(ii) TITLE OF INVENTION: Novel Branched Hybrid and Cluster Peptides Effective in Diagnosing and Detecting Non-A, Non-B Hepatitis

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: UNITED BIOMEDICAL INC.
(B) STREET: 25 Davids Drive
(C) CITY: Hauppauge
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 11788

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: M. Lisa Wilson
(B) REGISTRATION NUMBER: 34,045
(C) REFERENCE/DOCKET NUMBER: 9055

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 516-273-2828
(B) TELEFAX: 516-273-1717
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp Glu Leu His
1 5 10 15

Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile Gly Thr Val Leu Asp Gln
20 25 30

Ala Glu Thr Ala Gly
35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Phe Thr Phe Ser Pro Arg Arg His Trp Thr Thr Gln Gly Cys Asn Cys
1 5 10 15

Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met
20 25 30

Met Met Asn Trp Ser Pro Thr Ala
35 40

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Ile Leu Arg Lys Ser Arg Arg Phe Ala Gln Ala Leu Pro Val Trp
1 5 10 15

Ala Arg Pro Asp Tyr Asn Pro Pro Leu Val Glu Thr Trp Lys Lys Pro
20 25 30

Asp Tyr Glu Pro Pro Val Val His Gly Cys Pro Leu Pro Pro Pro Lys
35 40 45

Ser Pro Pro Val Pro Pro Pro Arg Lys Lys Arg Thr
50 55 60

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Ile Pro Phe Tyr Gly Lys Ala Ile Pro Leu Glu Val Ile Lys Gly
1 5 10 15

Gly Arg His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu
20 25 30

Ala Ala Lys Leu Val Ala Leu
35

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Pro Val Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr
1               5                  10                  15

Gly Ser Gly Lys Ser
            20
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Arg Glu
1               5                  10                  15

Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro Tyr Ile Glu Gln
            20                  25                  30

Gly Met Met Leu Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly Leu
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Arg Glu
1               5                  10                  15

Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro Tyr Ile
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg Gln Ala Glu
1               5                  10                  15

Val Ile Ala Pro Ala Val Gln Thr Asn Trp Gln Lys Leu Glu Thr Phe
            20                  25                  30

Trp Ala Lys His Met Trp Asn Phe
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 61 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg
1               5                  10                  15

Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly
            20                  25                  30

Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr
        35                  40                  45

Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg
    50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Asn Asp Arg Val Val Val Ala Pro Asp Arg Glu Ile Leu Tyr Glu Ala
1               5                   10                  15

Phe Asp Glu Met Glu Glu Cys Ala Ser Lys Ala Ala Leu Ile Glu Glu
            20                  25                  30
Gly Gln Arg Met Ala Glu Met Leu Lys Ser Lys Ile Gln Gly Leu
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Lys Lys Lys Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu
1               5                   10                  15

Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro Tyr
            20                  25                  30

Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe Lys Gln Lys Ala Leu
        35                  40                  45

Gly Leu
    50
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Arg Pro Asp Tyr Asn Pro Pro Leu Val Glu Thr Trp Lys Lys Pro
1 5 10 15

Asp Tyr Tyr Tyr Glu Pro Pro Val Val His Gly Cys Pro Leu Pro Pro
20 25 30

Pro Lys Ser Pro Pro Val Pro Pro Pro Arg Lys Lys Arg Thr
35 40 45

WE CLAIM:

1. A peptide composition comprising at least one branched peptide represented by the formula

$(peptide)_2X$

$(peptide)_4X_2X$

$(peptide)_8X_4X_2X$

$(peptide)_{16}X_8X_4X_2X$

wherein X is an amino acid or an amino acid analog having two amino groups and one carboxyl group, each group capable of forming a peptide bond linkage, and

said peptide moiety comprises at least one epitope which is specifically immunoreactive with NANBH-associated antibodies, wherein said peptide moiety comprises at least one cluster of from about 3 to about 20 contiguous amino acids selected from the group of sequences consisting of SEQ ID NOS: 1 to 10 (Pep3, Pep8, Pep11, Pep18, Pep25, IIH, IIID, V, VIIIE, PepA) and a sequence corresponding to one of said sequences which is from a corresponding region in a strain or isolate of HCV;

when said peptide moiety comprises two or more clusters, said clusters are joined by a linking group, said linking group being at least one natural amino acid, unnatural amino acid, or amino acid analog, and when said two or more clusters have sequences from a different one of the above sequences, said clusters can be joined directly or can be joined by said linking group; and

further wherein said peptide moiety comprises about 10 to about 100 amino acids.

2. The peptide composition of Claim 1 comprising a mixture of two or more of said peptides.

3. The peptide composition of Claim 1, wherein said peptide is conjugated to a carrier.

4. The peptide composition of Claim 1, wherein said cluster comprises from 5 to 9 contiguous amino acids.

5. The peptide composition of Claim 1 wherein said peptide moiety further comprises a segment of one of said sequences.

6. The peptide composition of Claim 1 wherein said sequence is the sequence designated as SEQ ID NO:3 (Pep11).

7. The peptide composition of Claim 6 wherein said peptide is C2A, C2B, C6A, C6B, C8A, C8B or C8C.

8. The peptide composition of Claim 1 wherein said sequence is the sequence designated as SEQ ID NO:4 (Pep18).

9. The peptide composition of Claim 8 wherein said peptide is C9A or C9B.

10. The peptide composition of Claim 1 wherein said sequence is the sequence designated as SEQ ID NO:6 (IIH).

11. The peptide composition of Claim 10 wherein said peptide is C3, C5A, C5B, C5C, 3KC5C, C7A, C7B, C7C or 3KC7C.

12. The peptide composition of Claim 1 wherein said sequence is the sequence designated as SEQ ID NO:7 (IIID).

13. The peptide composition of Claim 12 wherein said peptide is C1A or C1B.

14. The peptide composition of Claim 1 wherein said sequence is the sequence designated as SEQ ID NO:9 (VIIIE).

15. The peptide composition of Claim 14 wherein said peptide is C4, C10A, C10B, or 3KC10B.

16. A peptide composition comprising at least one branched hybrid peptide represented by the formula

$(peptide)_2X$

$(peptide)_4X_2X$

$(peptide)_8X_4X_2X$

$(peptide)_{16}X_8X_4X_2X$

wherein X is an amino acid or an amino acid analog having two amino groups and one carboxyl group, each group capable of forming a peptide bond linkage, and

said peptide moiety comprises at least one epitope which is specifically immunoreactive with antibodies against HCV, wherein said peptide moiety comprises a first sequence from one of the following sequences and one or more additional sequences, each from a different one of said sequences, wherein said sequence is selected from the group of sequences consisting of SEQ ID NOS: 1 to 10 (Pep3, Pep8, Pep11, Pep18, Pep25, IIH, IIID, V, VIIIE, PepA), a sequence corresponding to one of said sequences which is from a corresponding region in a strain or isolate of HCV, an analog of one of said sequences, and a segment of one of said sequences; and

further wherein said peptide moiety comprises about 10 to about 100 amino acids.

17. The peptide composition of Claim 16 wherein said sequences are the sequences designated as

SEQ ID NO:3, SEQ ID NO:7 and SEQ ID NO:9 (Pep11, IIID and VIIIE).

18. The peptide composition of Claim 17 wherein said peptide is H1.

19. The peptide composition of Claim 16 wherein said sequences are the sequences designated as SEQ ID NO:1 and SEQ ID NO:4 (Pep3 and Pep18).

20. The peptide composition of Claim 19 wherein said peptide is H3.

21. The peptide composition of Claim 16 wherein said sequences are the sequences designated as SEQ ID NO:3 and SEQ ID NO:4 (Pep11 and Pep18).

22. The composition of Claim 21 wherein said peptide is H4A or H4B.

23. The peptide composition of Claim 16 wherein said sequences are the sequences designated as SEQ ID NO:6 and SEQ ID NO:9 (IIH and VIIIE).

24. The peptide composition of Claim 23 wherein said peptide is H6A, H6B, H7 or 3KH7.

25. The peptide composition of Claim 16 wherein said sequences are the sequences designated as SEQ ID NO:7 and SEQ ID NO:9 (IIID and VIIIE).

26. The peptide composition of Claim 25 wherein said peptide is H2A, H2B, H2C, H2CK, H2D or H2DK.

27. A peptide composition comprising peptides 3KC10B, C9B, C6A and 3KH7.

28. A peptide composition comprising peptides 3KH7, C6A and C4.

29. A peptide composition comprising peptides C3, C4 and C2B.

30. A peptide composition comprising peptides C4, C9B, C6A and 3KH7.

31. A peptide of any one of Claims 1 to 30.

32. A method of detecting NANBH-associated antibodies which comprises using an effective amount of a peptide composition according to any one of Claims 1 to 30 in an immunoassay procedure.

33. A method of detecting NANBH or HCV infection which comprises contacting an effective amount of a peptide composition of any one of Claims 1 to 30 with a body fluid, tissue or tissue extract in an immunoassay procedure for a time sufficient to form a complex between said peptide composition and any antibody in said fluid, said tissue, or said tissue extract, and subjecting said complex to a detecting means.

34. The method of Claim 32 or 33 wherein said immunoassay procedure is an ELISA or a PHA procedure.

35. A kit for detection or diagnosis of NANBH or HCV infection comprising a first container adapted to contain the peptide composition of any one of Claims 1 to 30.

36. The kit of Claim 35 wherein said kit is an ELISA or PHA test kit.

37. An ELISA test kit for detection and diagnosis of NANBH or HCV infection comprising

(a) a container having a solid phase coated with the peptide composition of any one of Claims 1 to 30;

(b) a negative control sample;

(c) a positive control sample;

(d) specimen diluent; and

(e) antibodies to human IgG, said antibodies labeled with a reporter molecule.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08638

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 7/00; A61K 39/12; C12Q 1/70
US CL :530/324; 424/89; 435/5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324; 424/89; 435/5; 436/820

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,106,726 (Wang) 21 April 1992, entire document, especially col. 11, lines 10-27; col. 28, lines 50-66; and Example 7.	1-33, 35-37
Y	EP, A, 0,318,216 (Houghton et al.) 31 May 1989, see entire document.	1-33, 35-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
14 December 1993

Date of mailing of the international search report
JAN 03 1994

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08638

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.